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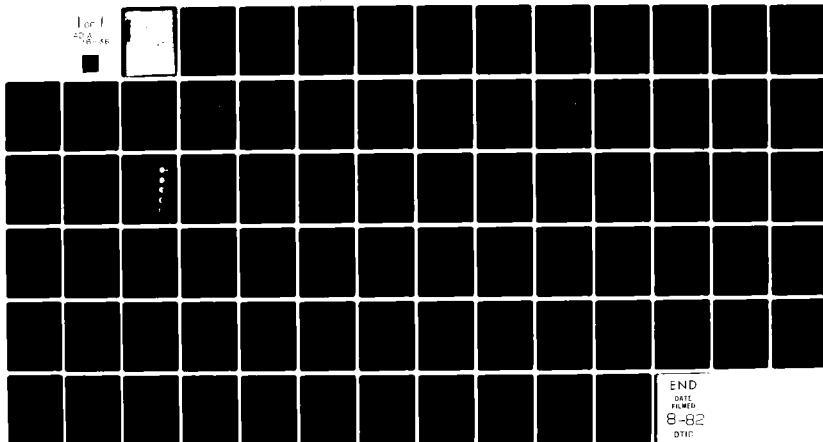
ARMY ENGINEER WATERWAYS EXPERIMENT STATION VICKSBURG--ETC F/G 6/6
TEST PLAN FOR THE LARGE-SCALE OPERATIONS MANAGEMENT TEST OF INS--ETC(U)
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The large-scale operations management test is being conducted in the state of Louisiana, using <u>Cercospora rodmanii</u> Conway, <u>Neochetina eichhorniae</u> Warner, and <u>Sameodes albiguttalis</u> (Warren) for the control of waterhyacinth. In-house and contractor groups will be collecting data on the project following the rationale set forth in this test plan. The plan includes a pilot study to determine whether <u>Cercospora</u> is capable of becoming epiphytotic on a large scale from the introduction of small quantities of inoculum, efficacy		

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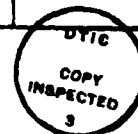
tests of a commercially prepared formulation of Cercospora, tests of effects of combinations of Cercospora - Neochetina and Cercospora - Sameodes, and supportive studies aimed at enhancing the effects of these agents. Provisions are also made for the extrapolation of results to the development of a management strategy for operational use of effective combinations of agents in areas where waterhyacinth is a problem.

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NOTE: The Large Scale Operations Management Test (LSOMT) outlined in this document is being conducted for the U. S. Army Engineer District, New Orleans, under the direction of the Aquatic Plant Control Research Program (APCRP). These demonstration projects are partly research and partly operational capability evaluations. The research portions address ARCRP program objectives set forth in the APCRP five-year plan (FY 77-FY 82) dated May 1978, Program Element I, Biological Control Technology.

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PREFACE

Funds for the research project described in this test plan are provided to the Aquatic Plant Control Research Program (APCRP) through the Department of the Army Appropriation No. 96X3123, "Operations and Maintenance General," by the U. S. Army Engineer District, New Orleans.

This document describes the plans for collecting and evaluating data for the various components of the overall Large-Scale Operations Management Test (LSOMT) to be conducted in the state of Louisiana, using insects and pathogens to control waterhyacinth. Dr. D. R. Sanders, Sr., is the overall team leader for the LSOMT. Mr. E. E. Addor is conducting the pilot field study, Mr. E. A. Theriot is responsible for the Cercospora efficacy tests, and Mr. R. F. Theriot is responsible for the field application tests.

Dr. Sanders and Messrs. Addor, R. Theriot, and E. Theriot of the Wetlands and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES, prepared the test plan under the direct supervision of Dr. H. K. Smith, Chief, WTHG, and under the general supervision of Dr. J. Harrison, Chief, EL, and Dr. C. Kirby, Jr., Chief, ERD. Manager of the APCRP at WES is Mr. J. L. Decell.

Commander and Director of the WES during the preparation of this test plan was COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) AND
METRIC (SI) TO U. S. CUSTOMARY UNITS OF MEASUREMENT

Units of measurement used in this report can be converted as follows:

<u>Multiply</u>	<u>By</u>	<u>To Obtain</u>
<u>U. S. Customary to Metric</u>		
inches	2.54	centimetres
feet	0.3048	metres
yards	0.914	metres
acres	4046.856	square metres
<u>Metric to U. S. Customary</u>		
millimetres	0.039	inches
centimetres	0.374	inches
square metres	10.76	square feet
grams/square metre	0.000205	lbs/square feet

TEST PLAN FOR THE LARGE-SCALE OPERATIONS MANAGEMENT TEST
OF INSECTS AND PATHOGENS FOR CONTROL OF WATERHYACINTH
IN LOUISIANA

PART I: INTRODUCTION

Background

1. Waterhyacinth (Eichhornia crassipes (Mart.) Solms.), a floating aquatic plant that covers extensive areas of water surface in the southeastern United States, poses a severe threat to navigation, fisheries, and recreational use of the waters. Although numerous methods have been proposed for the control of waterhyacinth, chemical herbicides (e.g. 2,4-D) are being used on an operational basis.

2. Because of a need to investigate methods of control other than chemical herbicides, and the promising results offered by the use of insects (e.g. Agasicles hygrophila Selman and Vogt) for the control of alligatorweed (Alternanthera philoxeroides (Mart.) Griseb.), searches were conducted in South America for potential biological agents for the control of waterhyacinth. During the period from the mid-1960's to the early 1970's, these explorations led to the selection of several candidate arthropods and fungal pathogens. The candidate organisms were screened for effectiveness as control agents for waterhyacinth and for host specificity in laboratory and greenhouse tests, and those organisms qualifying on both criteria were then selected for field trials. In the field trials, the potential effectiveness promised by the candidates in the greenhouse tests was only partially realized, but most candidate organisms produced at least some detrimental effects on waterhyacinth populations in the field. This led to the hypothesis that certain combinations of these organisms would produce a synergistic effect on waterhyacinth, which would result in greater control of waterhyacinth. Results of preliminary field experiments aimed at testing this concept indicated that increased control of waterhyacinth could be achieved by

the use of multiple agents and it was therefore considered appropriate to proceed with a Large-Scale Operations Management Test (LSOMT).

Purpose and Objectives

3. The purpose of this research project is to develop and demonstrate an operational capability for the use of selected combinations of insects and pathogens for the control of waterhyacinth. This document comprises the plan for a LSOMT for the introduction of selected insects and pathogens into a field operational environment for control of waterhyacinth in Louisiana.

4. The general objectives of the studies outlined in this test plan are:

- a. To determine the necessary and sufficient means for the establishment of effective populations of the selected organisms in the field in Louisiana for the control of waterhyacinth.
- b. To demonstrate the effectiveness of these organisms when used at an operational scale.
- c. To determine probable environmental limitations on the ability of these organisms to maintain effective populations in the field by natural regeneration.

Definition, LSOMT

5. LSOMT is the title used in the Corps of Engineers Aquatic Plant Control Research Program (APCRP) to designate a field test of an agent that has been proposed for the control of aquatic plants, to be conducted on a selected large area at a scale and in a manner representative of a full-scale field operations activity. Its purpose is to adapt basic laboratory and experimental research results to the field situation, and to integrate them into the operations programs. It differs from a purely scientific experiment both in scale and in the fact that a minimum

of experimental controls are imposed on the test area, hence, on the number of variables that may affect or be affected by the outcome of the experiment. It differs from a purely operational project in that the test area is carefully selected to satisfy certain requirements of the test design, and is carefully monitored over a sufficient period of time following application of the test treatments to determine whether the experimental agent or procedure is in fact cost-effective and environmentally acceptable at the scale of field operations.

6. The LSOMT is conducted cooperatively by research and operations personnel. This promotes a close working relationship between the two groups, thus ensuring that future laboratory work with the test agents or procedures will be done with full knowledge of the practical limitations and constraints of field operating conditions, and conversely, providing the operations personnel with an understanding of the theoretical limitations and special handling requirements of the test agents or procedures. Thus, the LSOMT provides training for operations personnel in the application of a new technique, while providing research personnel an opportunity to evaluate the technique in the operational context. It is a major step in the transfer of technology from the experimental to the operational context.

Rationale for Biocontrol with Multiple Agents

7. Biological control of plants refers to the use of herbivorous or pathogenic organisms to stress a pestiferous plant population. In general, one or more agents are sought that will adapt to the local environment and will adjust their populations rapidly in response to surges in the growth of the pest plant population. In some cases, however, it may be necessary to re-establish the control agent(s) periodically. A most important characteristic of a biocontrol agent is that it must not pose a threat to other species whose presence in the ecosystem is valued for any reason, and in particular it must not pose a threat to any economic species in areas where it may be introduced. Obviously, if an organism can be found that will effect satisfactory

control of the pest plant without posing a threat to other species, then the problem may be considered solved. However, if an agent is discovered whose effectiveness is erratic or inconsistent in time or space, two alternatives exist:

- a. The population of the control agent may be periodically re-established or artificially augmented, or
- b. Other organisms may be sought to supplement the effectiveness of the first.

Both of these alternatives apply to the biocontrol of waterhyacinth.

Plan Scope and Content

8. The remainder of this document focuses primarily on the description of a series of tests to be performed that addresses the general objectives of the LSOMT. It also includes a summary of previous studies leading to the conduct of the LSOMT, as well as basic information on the biology of the candidate biocontrol agents.

9. Preliminary studies leading to the selection of the organisms included in this test are described in Part II. Part III describes research to be performed as part of the LSOMT, including a pilot field study, a Cercospora spore formulation efficacy study, and a Cercospora spore formulation pilot field study. All of these studies are necessary to provide data requirements for planning and conducting a large-scale field application test of Cercospora, Neochetina, and Sameodes, which is also described in Part III. Certain fundamental questions relating to the biology and ecology of the organisms have been raised by the work done to date, and these questions are directly relevant to the efficient application and management of the organisms. These are considered to be fundamental to the concept and purpose of the LSOMT, but not essential to the conduct or satisfactory conclusion of it. Some of the more important of these questions are also addressed in Part III. Management implications of the test results are considered in Part IV. A proposed schedule of events is presented in Table 5. Basic background information on the organisms is summarized in Appendix A.

PART II: BACKGROUND STUDIES

Introduction

10. This part of the test plan is devoted to a description of work leading to the large-scale field tests with multiple agents, beginning after their release from quarantine, and certain individual preliminary field tests. This background work is divided into four reasonably distinct areas of research as follows: Lake Concordia experiment, distribution of Neochetina in Louisiana prior to the LSOMT, Cercospora formulation research, and Sameodes research. Brief descriptions of the organisms and sketches of the work leading to their selection as potential candidates for biocontrol of waterhyacinth are presented in Appendix A.

Lake Concordia Experiment

Objectives

11. The Lake Concordia experiment was designed to test the hypothesis that some mixture of candidate biocontrol agents would effect a greater degree of control on a waterhyacinth population than any one of the organisms used individually. For this experiment, a random-block factorial experimental design was used with the intention of identifying the relative contribution of each test organism to the results. Two insects, Arzama densa Walker and Neochetina eichhorniae (Warner), and two fungi, Cercospora rodmanii Conway and Acremonium zonatum (Sawada) Gams., were selected as the test organisms.* The rationale for these choices was based partly on the general observation that wounds are particularly good access sites for infection by fungi, and on the supposition that the insects would carry the fungal spores or mycelia to the feeding wounds. Specifically, it was hypothesized that feeding by the insects would stress the plants physiologically, thereby rendering them more susceptible to infection by the fungi. Furthermore, the

* Each of these organisms will hereafter be referred to by its generic name, unless otherwise noted.

movements of insects over and through the plants was expected to increase the rate of dispersal of the fungi, thus increasing the rate of infection. The selected insects were known to attack different organs of the waterhyacinth plant, so that their combined effect on the spread of the fungi and the intensity of the fungal infections should be more severe than with either used alone.

Methods

12. A random-block experiment was designed so that the four test organisms were represented separately and replicated four times in all possible combinations (16 possible combinations, including the untreated controls). The test plots (approximately 6 ft x 6 ft)* consisted of floating frames constructed of 4-inch-diameter aluminum pipe. These were anchored on open water near the north end of Lake Concordia in Concordia Parish, Louisiana, and filled with waterhyacinth plants collected from the population that grows naturally on the margins of the lake. Treatment plots were separated by a minimum distance of 20 ft, which was thought to be sufficient to prevent cross-contamination of the plots by migration or dissemination of the treatment organisms. The experiment was initiated in May 1975, and was continued through the growing season of 1978.

Results

13. The first and second year results of the Lake Concordia experiment have been reported (1,2,3). Reduction of plant biomass and height occurred on all test plots by the end of the second growing season, including the control plots. Cross-contamination of the control plots by the test organisms precluded obtaining statistically significant differences between treated and control plots. As a result, the relative contributions of the various organisms to the measured reduction could not be determined, nor could the most effective treatments.

* A table of factors for converting U. S. customary units of measurement to metric (SI) can be found on page iii.

14. Subjectively evaluated, the data suggested that the best results were obtained by a combination of Neochetina and Cercospora. However, that result could be entirely coincidental with the fact that these two organisms spread rampantly through the test area during the first season and persisted through the four years of observation. Arzama established only erratically on the plots to which it was introduced, but reappeared sporadically through the first and second seasons (appearing in the first season on 12 of the 32 plots to which it was not applied, including two of the controls). Acremonium established only poorly on the plots to which it was applied, diminished as the season progressed, and was not observed on any plot after the first season.

15. The waterhyacinths decreased in height by 60 percent and biomass by 70 percent during the second growing season. Plants in several of the plots did not recover at all after the third winter, and nearly all of the original test plots were empty by the end of the fourth growing season. Of those that still contained waterhyacinth plants, only a few contained more than 10 depauperate and deteriorating plants.

16. However, more interesting than the results in the test plots per se was the observation that both Neochetina and Cercospora had also spread throughout the natural population of waterhyacinth on the lake by the end of the second growing season, and are presumed to have been a primary factor in the conspicuous reduction of that population. This condition has persisted through the subsequent years of observation, with the waterhyacinth population on the lake now reduced to an acceptably low level of infestation.

Conclusions

17. While the results of this experiment did not permit definition of the effective combination of organisms or the relative contribution of each organism to the reduction of the waterhyacinth population on Lake Concordia, it is nonetheless suggested that at least some of these

organisms contributed significantly to the observed reduction. Although other factors (e.g. abnormally low winter temperatures) may have also been involved, it is apparent that Neochetina, Cercospora, and Arzama can persist on waterhyacinth under the environmental conditions that prevailed on Lake Concordia during the past few years, and that some combinations of these organisms contributed significantly in the reduction of the waterhyacinth population that occurred under those conditions.

Distribution of Neochetina in Louisiana

Objectives

18. Concurrently with the Lake Concordia experiment, biologists of the Louisiana Wildlife and Fisheries Commission (LWLFC) negotiated with appropriate agencies in Florida (USDA and Jacksonville District, CE) to obtain Neochetina for field release in Louisiana. The New Orleans District (NOD), CE, also participated in the initial establishment of Neochetina in Louisiana, especially in areas of major operational responsibility.

Methods

19. Adult Neochetina were collected from established field populations in Florida, shipped to Louisiana, and released in areas selected by LWLFC biologists to serve as sources of brood stock for future releases.

Results

20. Neochetina adapted readily to the waterhyacinth in Louisiana, and dense populations were soon established in the areas of original release. Subsequently, state and federal personnel responsible for aquatic plant control in Louisiana have redistributed Neochetina in large numbers from the initial populations (Figure 1), and the species is now widely distributed and fully naturalized in many localities throughout the state. The Neochetina population in some areas is very dense, but it remains sparse in other areas, for reasons yet unknown.

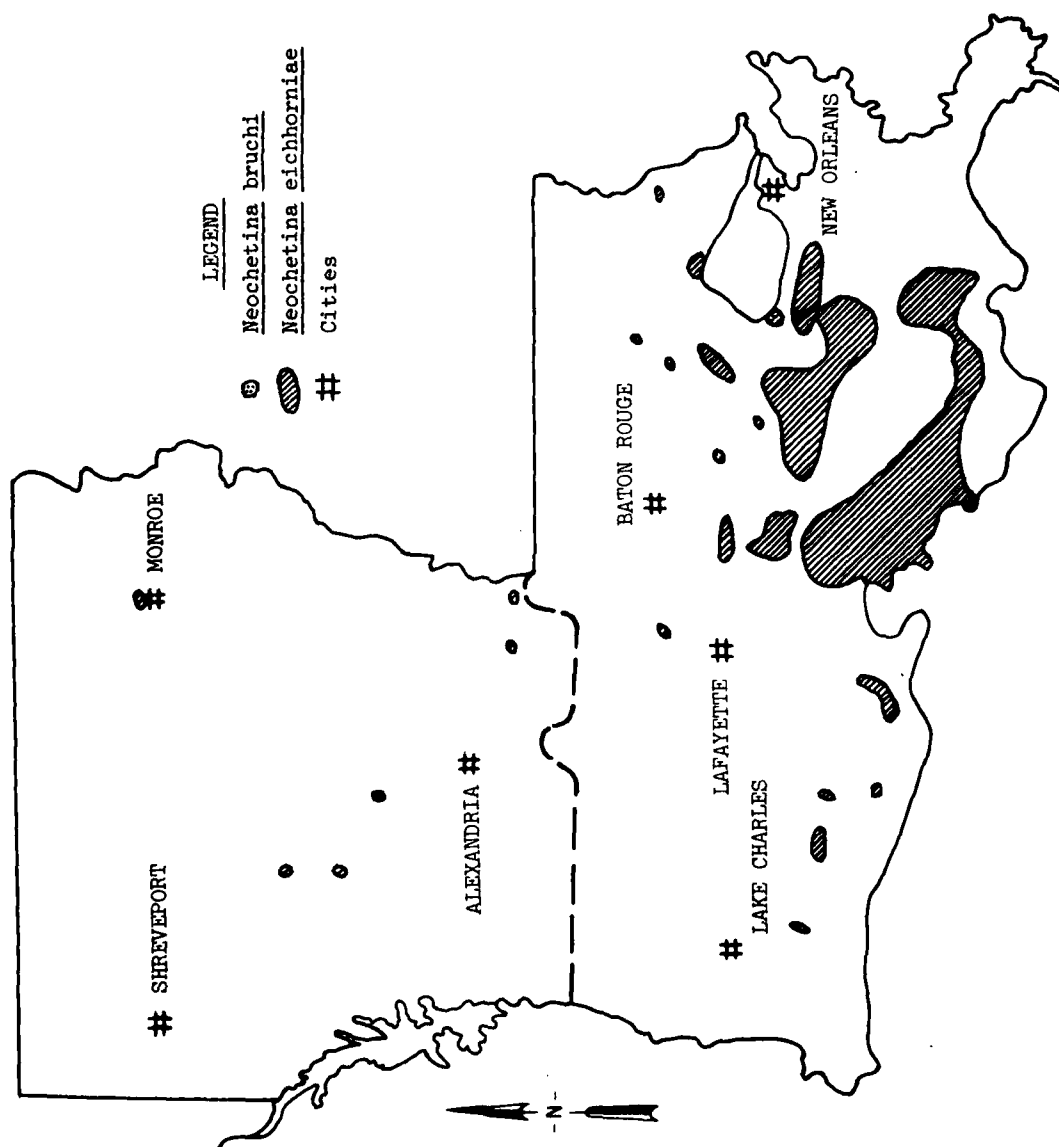


Figure 1. Map of Louisiana showing Neochetina sp. release sites

On the suggestion that slight, but significant, differences exist in the environmental adaptability and feeding patterns of N. eichhorniae and Neochetina bruchi Hustache (see Appendix A), the latter species has also been widely distributed in the state, and artificial redistribution of both species is continuing.

Cercospora Formulation Research

Objectives

21. After several years of research funded by the APCRP on Cercospora, the University of Florida was granted a patent for this species as a control agent for waterhyacinth. Arrangements were subsequently made with Abbott Laboratories, Chicago, Illinois, to develop a formulation suitable for mass application.

Methods and Results

22. The methods used by Abbott Laboratories are proprietary, but are based on traditional methods for mass production of antibiotics. Several proposed formulations developed by Abbott Laboratories have been tested on waterhyacinth by the University of Florida. As a result of these tests, Abbott Laboratories developed a spore formulation which meets the requirements for an effective biological control agent, and the formulation is ready for field testing.

Sameodes Field Study

Objectives

23. After Sameodes albiguttalis (Warren) was cleared for release from quarantine (see Appendix A), a study funded by the APCRP was initiated in 1977 by the USDA Aquatic Plant Management Laboratory, Fort Lauderdale, Florida, to determine the requirements for field establishment of the species. A second major objective is to determine the rate of population development and dispersal from the release sites.

Methods

24. Various combinations of the different stages of the life cycle of Sameodes were placed in eighteen sites in a band across southern Florida. Also, plants containing mixtures of eggs, larvae, and pupae were placed in some of the test sites. All sites are being monitored for establishment, population development, and rate of dispersal.

Results

25. Preliminary results indicate that successful establishment of Sameodes can be effected by the use of first instar larvae placed directly on the leaves or by the placement of infected plants in test sites. However, no single method has proven effective in every case. The rate of population development is somewhat slower than originally expected, and the species has been found only a few hundred yards away from the original release site (4).

Conclusions and Recommendations

26. Considering the results of the Lake Concordia experiment and other research described above, it was concluded that a large-scale field test using combinations of organisms for the control of waterhyacinth should be conducted. Consequently, an LSOMT was designed on the following precepts:

- a. Cercospora would be introduced to selected sites, including some with and without Neochetina already present. Three different methods of treatment application to be explored: (1) cultured live mycelium - prepared as a spray, which was the method used on Lake Concordia; (2) transplant - defined as the introduction of diseased plants into target waterhyacinth populations; and (3) commercial formulation - the formulation resulting from the Abbott Laboratory research, applied in the most appropriate manner. The relative effects and epidemiology of the various treatment methods would be compared.

- b. Neochetina would be distributed to selected sites where it was not already present, and its rate of population increase, dissemination, and effects on waterhyacinth would be determined.
- c. Neochetina and Cercospora would be introduced simultaneously on selected sites, and the development of their combined populations and effects would be determined.
- d. Arzama would be artificially disseminated if improved methods could be developed for mass culturing of larvae. An effort would be made to find one or more test sites with significant Arzama populations. In either case, effects of Arzama on waterhyacinth would be studied.
- e. Acremonium would be considered, only to the extent that field observations would specifically include a search for evidence of this disease on the test sites.
- f. All other commonly occurring organisms on the field sites would be similarly recorded whether or not previously observed on Lake Concordia.
- g. Sameodes albiguttalis would be incorporated into the tests, subject to its availability from quarantine.

PART III: LARGE SCALE OPERATIONS MANAGEMENT TEST

27. At approximately the same time that a decision was made to conduct a LSOMT of insects and pathogens for the control of waterhyacinth (paragraph 26), the NOD approached the Office of Chief of Engineers and the WES requesting assistance in the development of a biological system for the control of waterhyacinth within the District. In spite of large, effective waterhyacinth control programs being conducted by both the NOD and the LWLFC, a massive waterhyacinth problem persisted in Louisiana, due to the presence of extensive backwater areas that served as nursery grounds for waterhyacinth. It was thought that implementation of a biological control system in the backwater areas would impact the reproduction rate of waterhyacinth sufficiently to provide relief from the problem. Subsequent discussions and planning resulted in a decision to conduct a LSOMT of insects and pathogens for the control of waterhyacinth in the state of Louisiana with funds provided by NOD. This portion of the test plan defines a series of tests that comprise, and are essential to, the overall conduct of the LSOMT: (a) pilot field study; (b) efficacy test of commercial formulation of Cercospora; (c) small-scale field test of commercial formulation of Cercospora; (d) large-scale field application of commercial formulation of Cercospora; and (e) field release of Sameodes.

LSOMT Pilot Field Study

28. To determine whether Cercospora rodmanii would become established and thrive in the environmental conditions present in NOD, a pilot field study was initiated in the 1977 growing season. The study was initiated on the premise that the successful establishment and rapid development of Cercospora to epiphytotic levels would preclude the need for a long-term, expensive research program required for the development of a Cercospora formulation that could be applied in a manner similar to a herbicide.

Objectives

29. Objectives of the pilot field study are:

- a. To test certain proposed field procedures for transporting and disseminating the test organisms.
- b. To test the ability of Cercospora to develop into an epiphytotic from the application of small quantities of inoculum.
- c. To develop appropriate monitoring and observation requirements for the large-scale test sites.
- d. To acquire familiarity with the basic phenology of the waterhyacinth on a large-scale in the test area.

Methods

30. The initial field reconnaissance in June 1977 covered much of Louisiana south of a line from New Orleans to Baton Rouge to Lake Charles. Sites initially visited were recommended by personnel of the NOD and LWLFC as possibly suitable for the proposed LSOMT tests. They included sites where Neochetina had been introduced one to three years previously, and on which the insects were then present in various densities. None of these sites had been routinely sprayed with herbicides by either agency for waterhyacinth control, and none were scheduled to be sprayed. Following site selection (Figure 2), the sites were evaluated and classified in terms of their hydromorphic and geographic characteristics.

31. Following the site characterizations, initial treatments of Cercospora and Neochetina were made on three sites on 21-23 June 1977, both by the transplant method. The Cercospora-infected plants were taken from Lake Concordia plots, and Neochetina-infested plants were collected from a site with a well-established Neochetina population near Sorrento, Louisiana. Additional sites were inoculated with Cercospora on 1-3 Aug 1977 with live cultured mycelium supplied by the University of Florida, and on 30-31 August 1977 with additional transplants from Lake Concordia. In all cases, "treatment" consisted of a spot application (approximately 0.25 m^2 in size) of 10-20 Cercospora-infected plants at

one or two spot locations on sites ranging in size from a few to several hundred acres. Additional sites, including some in the northern portion of the state, were treated in 1978 using the same application methods. All treatment sites included in the pilot study are shown in Figure 2.

32. Following treatment, the sites were inspected at approximately one-month intervals through the 1977 and 1978 growing seasons. Notes were kept on the general condition of the plants, including height, growth stage, and vigor, symptoms of disease and presence of activity of insects, and general site conditions (e.g. water quality, depth direction, and rate of flow). Specimens were collected periodically for culture tests to verify the presence of Cercospora.

33. The above observations were extensively documented with color photographs. To ensure that variations in visible site characteristics through time would be reasonably documented on the photographs, a sketch map was drawn for each observation point and its immediate environs, and the location and direction of every photograph was recorded. At each location, selected scenes were photographed routinely at each visit.

Results

34. On all test sites where significant reductions in the waterhyacinth population were observed during the second season after inoculation, either Cercospora or Neochetina or both were present. On several sites, the reduction of waterhyacinth occurred after Cercospora was introduced. However, the waterhyacinth population also declined significantly at one site that had a high population density of Neochetina. The organisms that appear to be significantly associated with these results are Cercospora, Neochetina, and Arzama. However, the relative contribution of each organism to the results was not determined. Abnormally low winter temperatures during the test period could have been a major factor contributing to the decline of waterhyacinths in the test sites. Nevertheless, the evidence suggests that these organisms significantly impacted the growth of waterhyacinth in the sites.

35. Based on data already obtained in this study, the following observations concerning the test organisms have been made:

- a. Neochetina establishes readily in most environments within the study area, and it commonly develops high population densities very rapidly at the release point. However, it apparently has a natural tendency to aggregate and does not disseminate rapidly, except where plants are free to drift. Neochetina adults usually feed on newer waterhyacinth tissues, while Cercospora normally produces significant infection on moderate to older tissues.
- b. Cercospora has been positively identified from some sites to which it was introduced, including some in which the transplant method was used. The species is easily established by this method, and spreads rapidly through the waterhyacinth population. A deleterious effect on the infected waterhyacinth population, however, is usually not manifested until at least the second season. This slow rate of development of a stressed waterhyacinth population is directly related to the response of both Cercospora and waterhyacinth to the cool temperatures common in the spring and fall seasons. During these seasons, Cercospora growth is favorable and the buildup of inoculum is rapid. During the same period of time, the growth rate of the waterhyacinth population is lower than in the summer months. The net result is an increased impact of Cercospora on waterhyacinth in the spring and fall, and a decreased impact during the summer. Growth from the originally applied inoculum in the fall of 1977 resulted in sufficient secondary inoculum to permit Cercospora to spread to adjacent areas within the site. However, there was no massive buildup of inoculum prior to the onset of winter. During the following spring, inoculum buildup was significant in plant tissues infected the previous fall, and inoculum spread to other plants.

After a period of relative inactivity during the summer, Cercospora began another period of active growth in the fall. By that time, there was sufficient inoculum present on the existing waterhyacinth plants to significantly impact the population.

- c. Arzama occurs sporadically in time and space throughout the test area, and it sometimes becomes locally abundant. However, its activity is restricted to large, lush plants, and although it is quite destructive, the Arzama-infested waterhyacinth populations may recover readily. Arzama produces only two generations per year, Arzama-infested waterhyacinth plants are only stressed when the larvae are active. During other stages in the life history of the species and at times when parasites and predators limit the population, waterhyacinths are stressed very little by Arzama. Thus, the primary value of naturally occurring Arzama as a control agent for waterhyacinth lies in its temporary or seasonal effects on large plants. Research on procedures for efficient artificial manipulation of the insect populations has not yet yielded satisfactory results. Until such procedures are developed, the use of Arzama as an agent for the control of waterhyacinth will be limited to areas where large, natural populations of the insect sporadically occur.
- d. Acremonium has been observed on some of the sites, but it has been uncommon, never abundant, and apparently not particularly destructive to the infected plants. It appears that under the climatic conditions prevailing in the test area during this period of observation, Acremonium is unlikely to be of significance as a bio-control agent in Louisiana.

- e. Orthogalumna and Tetranychus are ubiquitous on waterhyacinth in the test area. The former tends to be most abundant on plants of intermediate to low vigor, while the latter occurs almost exclusively on very lush plants. Although ubiquitous, the contributions of the species to the decline of the waterhyacinth populations observed during the past two seasons or previously on Lake Concordia appear to have been insignificant. Given the demonstrated potential of other organisms now available for biocontrol of waterhyacinth, any attempts at artificial manipulation of these species on a large scale seem unwarranted. Furthermore, Tetranychus is not host specific, and often attacks a broad range of economically important plant species.
- f. Caterpillars and grasshoppers are locally abundant on waterhyacinth in the test area, but their consumption is insufficient to impact the plants. Their feeding is restricted to the expanded pseudolamina and this activity apparently has little or no effect on the general vigor of the plant.

Preliminary Conclusions

36. It is concluded that at least partial control of waterhyacinth in Louisiana by biocontrol agents is not only possible, but probable. This does not of course preclude a need for continued spray or mechanical operations for small-scale local maintenance or periodic maintenance on a larger scale when weather factors favor the waterhyacinth. Continued dispersal of Neochetina and Cercospora would appear to be useful and cost effective, in view of the remarkably low cost and simple logistics of the transplant technique(s). However, the rate at which the waterhyacinth declines will be site dependent, due to as yet undefined variations in environmental conditions from site to site. Also, it has not yet been determined that the technique is effective in all circumstances.

Continuation of Pilot Study

Objectives

37. The objectives of this work are:

- a. To determine the ability of Cercospora to become epiphytotic on waterhyacinth populations previously treated with Cercospora.
- b. To define phenological attributes that may affect the operational use of biological agents for the control of waterhyacinth in Louisiana.

Methods

38. Observations on the previously selected sites throughout the state will be continued as in the past two seasons (paragraphs 32 and 33). However, certain quantitative measurements will be made in addition to the visual estimates of infestation or infection. Neochetina population densities will be estimated by the use of a feeding scar index (5). The number of Neochetina feeding scars on each leaf of twenty randomly selected waterhyacinth plants from each site will be determined by the use of the following feeding scar classes:

<u>Feeding Scar Class</u>	<u>Range of Feeding Scar Class</u>
0	0
1	1-25
2	26-50
3	51-100
4	100-200
5	>200

The average number of Neochetina feeding scars per plant will be calculated and used as an indication of relative Neochetina population densities.

39. To determine the infectivity of Cercospora on the waterhyacinth in the test sites, Cercospora damage per leaf will be based on a rating scale developed by Dr. K. E. Conway and associates at the University of

Florida (6). The damage will be rated on a scale of 0-9 where 0 refers to no apparent infection on the leaf and 9 indicates a dead submerged leaf blade and petiole. The values of between 1 and 8 correspond to increasing coverage of the leaf blade by the pathogen (Table 1).

40. Selected environmental conditions (Table 2) will be monitored at certain sites. An attempt will be made to pair previously treated sites with analogous untreated sites for these measurements. Measurements will be made by instruments installed at the site or carried to the site on a prescribed schedule. The specific sites for instrumented observations will be selected to represent variations in hydrological characteristics, hydromorphology, topography, and adjacent vegetation or land-use patterns.

Schedule

41. Visual observations will continue on a monthly basis for the duration of the LSOMT. The average number of Neochetina feeding scars per plant and Cercospora infectivity will be estimated quarterly. Most environmental monitoring will be conducted in May of each year. However, water temperature, depth, pH, and dissolved oxygen will be monitored monthly.

Reports

42. A preliminary report covering the first two years of the pilot studies will be submitted by 1 July 1979. A final report will be submitted not later than six months following cessation of the monthly observations.

Cercospora Spore Formulation Efficacy Test

Purpose and Scope

43. The purpose of this preliminary, small-scale outdoor test of the LSOMT is to demonstrate the effectiveness of the Cercospora formulation produced by Abbott Laboratories for the control of waterhyacinth.

Table 1.
Rating Scale System for Damage to Leaves of Waterhyacinth by *Cercospora Rodmanii*

Numerical Rating Symptoms

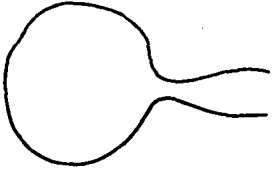
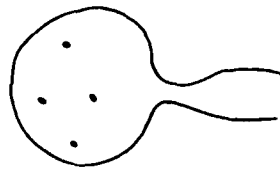
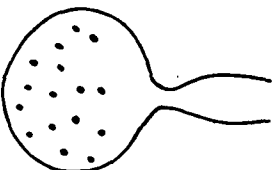
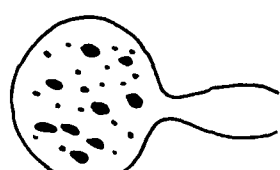
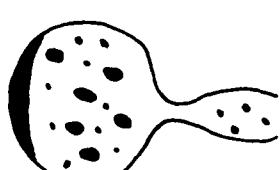
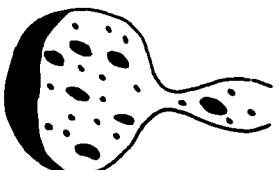




2	No spots on leaf or petiole.		1	1 to 4 spots on leaf, no petiolar spotting.		2	Less than 25 percent of leaf surface with spots, no coalescence or petiolar spotting.		3	Less than 50 percent of leaf surface with spots, some coalescence, no petiolar spotting.		4	Less than 25 percent of leaf surface with spots, coalescence, some tip dieback and petiolar spots.		5	Less than 50 percent of leaf surface with spots, coalescence, 10 percent tip dieback, petiole spotting.		6	Less than 75 percent spots, coalescence, 30 percent tip dieback, increasing petiole spotting.		7	Greater than 75 percent spots, coalescence, 60 percent tip dieback, coalescing spots on petiole.		8	Dead leaf blade, petiole green, but heavily spotted.		9	Dead leaf blade and petiole (submerged).	
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Table 2. Site Environmental Descriptors to be
Measured on Selected Sites

A. Water Factors:

pH
Salinity
Hardness
Nitrogen (Available)
Phosphorus (Available)
Flow: direction and rate
Temperature 2-4 inch below surface

B. Atmospheric Factors:

Temperature: at 3/4 the canopy height
Relative humidity: at 3/4 the canopy height
Wind: direction and speed

C. System Hydromorphology

Channel cross sections
Topographic setting
Adjacent land use or vegetation type

This portion of the test plan describes an experimental design for an efficacy study of the spore formulation of Cercospora on waterhyacinth.

Objectives

44. The specific objectives of the test are:

- a. To determine the infectivity and effectiveness of the spore formulation of Cercospora in controlling waterhyacinths.
- b. To establish treatment rates to be used in the LSOMT.

Experimental Design

45. Schedule. Data specified in paragraphs 50 and 51 will be collected on the second, fourth, and seventh day after treatment application. After the first week, sampling will be conducted on a weekly basis for six weeks following the treatment application date, after which the data will be analyzed and a report will be prepared. The following is a schedule of the test milestones:

Establish test tanks	15 Mar 1979
Application of pathogen and data collection	2 Apr 1979
Data collection, second day	4 Apr 1979
Data collection, fourth day	6 Apr 1979
Data collection, seventh day	9 Apr 1979
Data collection, second week	16 Apr 1979
Data collection, third week	23 Apr 1979
Data collection, fourth week	30 Apr 1979
Data collection, fifth week	7 May 1979
Data collection, sixth week	14 May 1979
Initiate report	15 May 1979
Complete final report	1 Jul 1979

46. Formulation and treatment rates. The liquid spore formulation of Cercospora produced by Abbott Laboratories will be applied at three rates: 0.5, 1.0, and 1.5 times the rate suggested by Abbott Laboratories.

In addition, there will be two sets of controls: one to be sprayed with the carrier substrate present in the Cercospora spore formulation, and the other which will not be sprayed. Each treatment rate and control will be replicated three times.

47. Treatment tanks. A total of 15 tanks will be utilized in this study. To minimize cross-contamination, the tanks will be separated in strategic locations on the WES grounds by a minimum distance of 300 ft. To preclude a toxic reaction of the waterhyacinths to the zinc content in the walls of the tanks, the oval galvanized tanks (2 ft x 6 ft x 2 ft) will be lined with two layers of polyethylene. Each tank will be filled with tapwater to approximately equal depths and maintained at that level throughout the test period. Equal amounts of nutrient solution will be added to the tanks to ensure that nutrient deficiencies are not limiting to the growth of the waterhyacinths. At the time of the tanks establishment, the pH will be adjusted to 6.5 in each tank and maintained within a range of 6.0 to 7.0 throughout the test period.

48. Plant placement and tagging. Twelve Stage-II waterhyacinth plants (7) will be added to each tank on the date scheduled for the test tanks (paragraph 45). Before the plants are added to the tanks, all dead or dying leaves and all daughter plants will be removed. On the date of treatment application, six plants will be randomly chosen from each tank and weighed to determine biomass. The remaining six plants will constitute the test plants. Six waterhyacinth plants will be sufficient to cover approximately 1/3 of the surface area of the tank, which will prevent crowding and provide opportunity for the production of daughter plants through stolen formation. In this manner, it will be possible to determine effects of stress by the pathogen on the ability of the plants to reproduce asexually. To measure this effect, the newest emergent leaf of each plant will be tagged on the day of treatment application. By so doing, it will be possible to discriminate between the original plant tissue that receives direct application of the pathogen and new, untreated tissue. The original tissue of each

plant will consist of the tagged lead and all leaves distal to it, while the new plant growth will consist of all plant tissues proximal to the tagged leaf and all untagged daughter plants.

49. Treatment application. The formulation of Cercospora will be applied to the tanks by a hand-held sprayer at the rates specified in paragraph 46. After a review of the temperatures recorded in the area for the past three years, 2 April 1979 was selected as the target date for treatment. This date was selected because damaging frosts have not occurred on or following this date in the past three years. In addition, the mild temperatures that normally occur in April favor pathogen development, and waterhyacinths grow at a much slower rate than during the peak growth period later in the season.

Data Collection

50. Physical. On the dates designated for data collection (paragraph 45), visual observations of each treatment tank will be made. The water temperature and the pH will also be noted and the pH will be adjusted if necessary (paragraph 47). An automated weather station will be set up in the test area to monitor the air temperature, humidity, wind direction and force, and the water temperature at root level in one of the test tanks on a continuing basis throughout the test period.

51. Biological. The stage of growth of the plants (7) and the presence or absence of flowers will be noted, and both color and color infrared photos will be taken of each treatment tank. All original plants in each tank will be examined on each sampling date. Data to be collected will include an average of disease damage per leaf for both original and new plant tissues, the number of emergent leaf blades per plant, the number of dead leaves per plant, and the height of the plants. Length of the root system will be measured on the date of application and again at the end of the test period. The total number of new daughter plants per tank will also be recorded. The biomass of the plant population in each tank will be determined on the last sampling

date. The infectivity scale described in paragraph 39 will be used to monitor the direct effects of Cercospora on waterhyacinths in the test plots.

Data Analysis

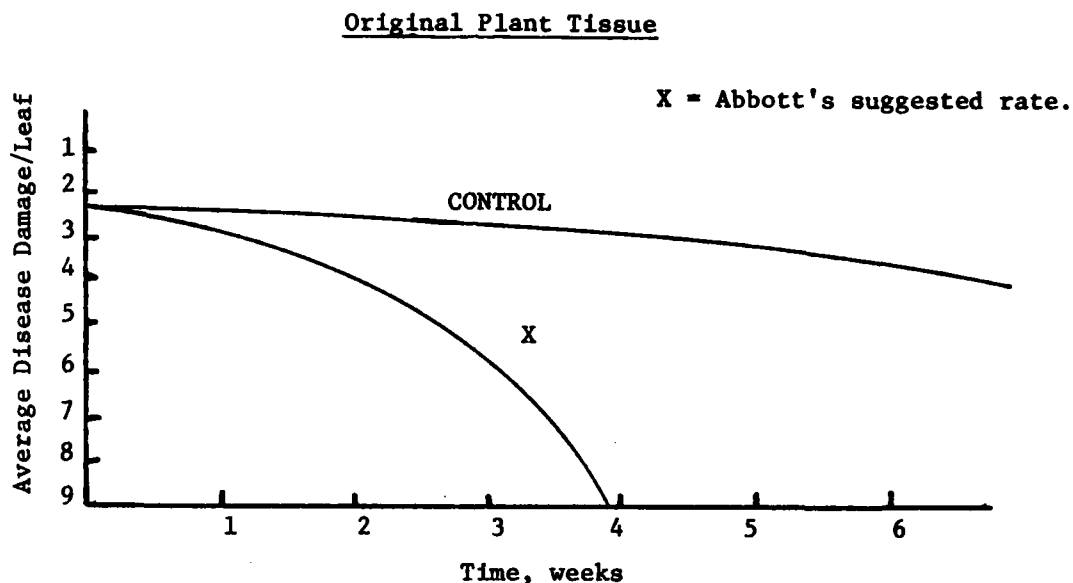
52. All data obtained will be entered into the computer for analysis via the Statistical Analysis System (SAS). By use of the SAS, analysis of variance (ANOVA), multiple-range tests, probit analysis, and/or graphics will be performed on the various data components to determine significant differences in the effect of the treatment rates tested.

53. Physical. Values for water and air temperatures and relative humidity will be plotted against time to depict the total variation in these environmental parameters during the experiment. These graphics will provide an indication of possible effects on the plant populations in the tanks due to weather conditions during the test period.

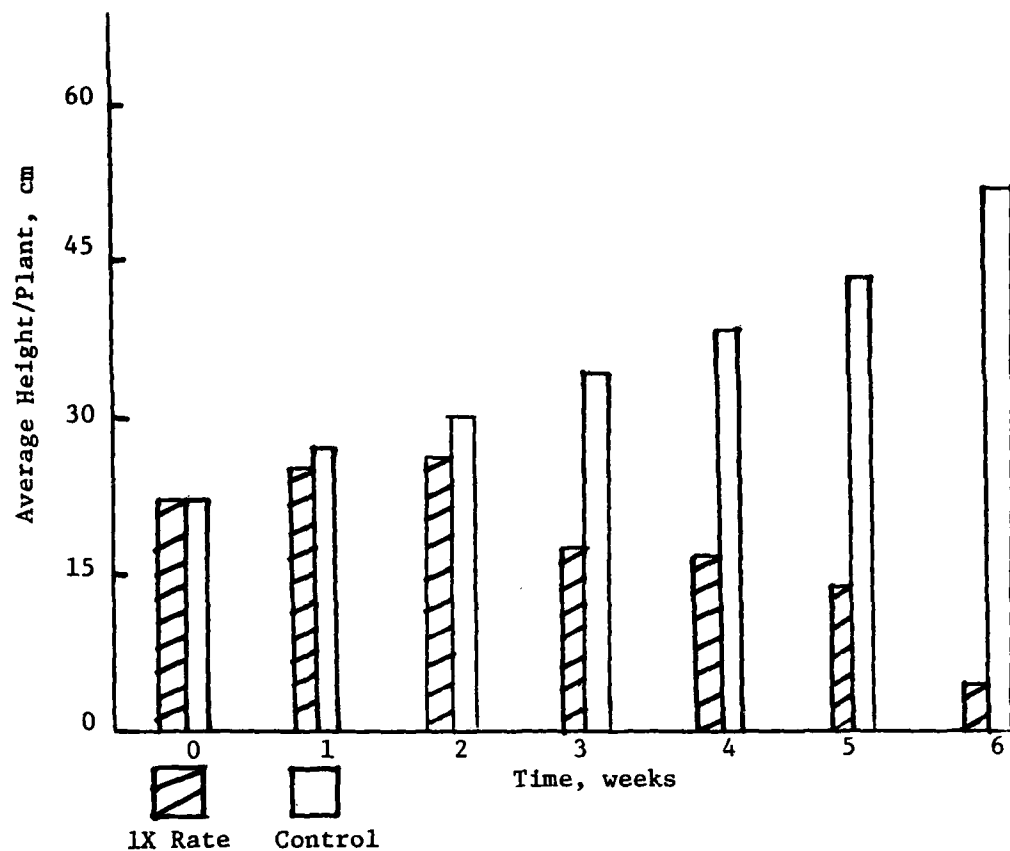
54. Biological. The disease damage per leaf for original and new plant tissue, the number of emergent leaf blades per plant, the number of dead leaves per plant, the height per plant, and the number of daughter plants per tank will be averaged for each tank and treatment rate and the appropriate statistical procedures will be performed for each date of data collection. Root lengths of the original plants and the biomass for the entire living plant populations will be determined for each tank on the last sampling date and compared to the values obtained on the date of application. The values will provide an indication of the impact of various Cercospora formulation treatment rates on each plant characteristic and will determine if observed differences are statistically significant.

Data Portrayal

55. Effects of the various treatment rates of the spore formulation of Cercospora on waterhyacinths will be portrayed both graphically and in tabular form. The following graph is an example of the type of relation that can be shown in this manner:



This type of graph will also be used for showing effects on new plant tissues, number of emergent leaf blades per plant, and the number of dead leaves per plant. Plant height, which has been shown to be a good indication on the pathogen effect on waterhyacinth, will be portrayed in the following manner:



Report

56. Upon completion of data collection and analysis, a report will be prepared for publication by the WES.

Cercospora Spore Formulation Pilot Field Study

Purpose

57. The primary purpose of the Cercospora spore formulation field application study is to test methods and equipment for use in applying the spore formulation of Cercospora in the large-scale field application test. A secondary purpose is to compare efficacy of the spore formulation to that of the wet mycelial formulation originally used by Dr. K. E. Conway et al. in infectivity tests (8).

Objectives

58. The specific objectives of the small-scale field test are:
- a. To determine the method of application and equipment to be used in the large-scale field application test.
 - b. To verify the infectivity of the spore formulation of Cercospora on waterhyacinth in the field.
 - c. To compare the effectiveness of the spore formulation to that of the wet mycelial formulation.

Experimental Design

59. Schedule. The following is a schedule of the test milestones:

Plot establishment	5 Sep 1979
Pre-treatment data collection	19 Sep 1979
Application of pathogen	19 Sep 1979
Data collection, first week	26 Sep 1979
Data collection, third week	10 Oct 1979
Data collection, sixth week	7 Nov 1979
Initiate report	8 Nov 1979
Completion of report	21 Dec 1979

60. Formulation and treatment rates. The liquid spore formulation of Cercospora will be applied at a single treatment rate, which will be determined following evaluation of results of the spring efficacy test (paragraphs 43-56). Also a wet mycelial formulation provided by

Abbott Laboratories will be applied at a rate of 100 g/m^2 for comparison of effects (8). The substrates used in the preparation of each formulation will be applied to control plots. Each treatment will be replicated twice.

61. Treatment plots. Treatment sites will be selected in south Louisiana because the probability of damaging frost prior to completion of the experiment is lower in the southern portion of the state. Twelve plots (12 ft x 12 ft) will be established at sufficient distances from each other to minimize cross-contamination. Determination of plot spacing will be influenced by results of the efficacy test described in paragraph 46. Criteria to be used in site selection include:

- a. Presence of a dense population of waterhyacinth.
- b. Location that will not be sprayed with herbicides.
- c. Locations that are readily accessible by boat for application and data collection.

62. Treatment application. The spore formulation will be applied to two plots each by use of raindrop, mister, and hollow-cone type nozzles. Each nozzle type will be used to spray two test plots. The wet mycelial formulation will be applied to two test plots using the mister type nozzle, which was shown to be the most effective type for application of this formulation (9). The different rates and controls will be applied to the test plots by a hand-held spray system from a boat. The date of application, specified in paragraph 59, was chosen after a review of the daily temperatures for the area in the past three years. The milder fall temperatures favor pathogen development, and waterhyacinths grow at a much slower rate than during the peak growth period earlier in the season.

Data Collection

63. Physical. During the test period, an automated weather station will be located on the treatment sites. The instrumentation will be used to monitor the water temperature at root level, relative humidity

in the waterhyacinth canopy, air temperature, and wind direction and velocity at hourly intervals for the duration of the test. On dates designated for data collection, both color and color-infrared photos of the test plots will be taken and pH, temperature, and salinity of the water in each plot will also be measured.

64. Biological. On each sampling date, eight plants will be randomly chosen from each treatment plot for detailed observation, using the following procedure. The test plots will be divided into four equal quadrants and two plants will be selected from each quadrant by throwing a 4 inch styrofoam sphere into each quadrant on a randomly chosen compass setting and removing the two waterhyacinth plants nearest the sphere (5). The plants from each treatment plot will be placed in a plastic bag and stored for laboratory analysis. Data to be collected from the selected waterhyacinth plants will include the disease damage per leaf, plant height, and length of the root system (paragraph 54). In addition, an estimate of the standing crop of the waterhyacinth population will be determined on the day of treatment application, and again on the final day of data collection using the method outlined by Forno and Bourne (10). This method requires that the length of each petiole be determined in four 0.25-m^2 sample areas per test plot and placed into one of five classes: 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, and >60 cm. The total number of petioles in each class will be multiplied by an average value of the dry weight per leaf for each class and added to the values obtained for the other classes. This method has been proven to be correct within 10%, and is a quick and accurate estimation for use in field studies.

Data Analysis

65. All data, both physical and biological, will be analyzed as outlined in the Cercospora efficacy study (paragraphs 52-54). The average values of the standing crop estimations of each treatment for pre- and post-treatment values will be analyzed using analysis of variance (ANOVA).

Data Portrayal

66. The analyzed data will be portrayed in the same manner as outlined in paragraph 55 of the Cercospora efficacy study.

Report

67. After the data have been collected and analyzed, a final report will be prepared by 21 December 1979 for publication by the WES as a miscellaneous paper.

Large-Scale Field Application Test of Biocontrol Agents

Purpose and Scope

68. The overall purpose of this part of the LSOMT is to obtain the data necessary to determine the feasibility of using selected combinations of insects and pathogens to control waterhyacinth on an operational basis in Louisiana. This portion of the test plan defines the requirements for placing the biocontrol agents into the test environment, collecting the necessary data, and extrapolating the results for management use at the operational level.

Rationale and Approach

69. Previous small-scale studies have demonstrated that specific levels of individual biocontrol agents (e.g. Cercospora (11)) or combinations of these agents (paragraph 15) can significantly stress waterhyacinth. Intensive studies (see Appendix A) have revealed that these organisms are strongly host specific and pose no direct threat to other organisms in the environment. Any effect on the associated flora and fauna in the test sites will be associated directly with the anticipated decline of waterhyacinth and will be viewed as no worse than the effect due to the decline of waterhyacinth by any other control method (e.g. chemical or mechanical). Consequently, monitoring for possible effects of the biocontrol agents on nontarget components of the ecosystem (e.g. fisheries, waterfowl, mammals, etc.) will be excluded from this test.

70. Although they are known to be safe for release into the environment, there have been no large-scale studies of the effects of mass-applied biocontrol agents on waterhyacinth, nor has there been a demonstration of the ability of these agents or combinations of agents to effectively control waterhyacinth on a large scale. Consequently, much of the data needed for development of an operational plan for the use of organisms for the control of waterhyacinth are lacking. For example, no data are available describing the effects of climate on the efficacy of Cercospora, no studies have been conducted on the interactive effect of Cercospora and Sameodes, and the requirements for the large-scale application of Cercospora are unknown. Answers to these and many other questions must be obtained before a manual can be developed for the use of these biocontrol agents by operations personnel.

71. Following the above rationale, the approach to the large-scale field application test will be to select test sites that meet certain basic criteria, effect the desired treatments on the test sites, and monitor the effects of treatments on waterhyacinth in the test sites for a sufficient period of time to obtain the data required for development of an operations manual.

Objectives

72. Using the above rationale and approach, the specific objectives of the large-scale application test are:

- a. To determine the most effective combination of biocontrol agents for the control of waterhyacinth in Louisiana.
- b. To develop the framework of an operational system for the routine use of biological agents for control of waterhyacinth.
- c. To assess the cost of implementation of the resulting operational system.

Test Area and Types

73. The state of Louisiana has been selected as the test area.

Louisiana has more than four million acres of fresh water, nearly all of which is potentially infestable by waterhyacinth. The state is drained by nine major river basins (Figure 3), all of which have substantial infestations of waterhyacinth.

74. Two basic types of field tests will be conducted, varying according to the size of test sites and degree of replication. In the replicated test, the efficacy of various treatment combinations of Cercospora and Neochetina and the effect of climate on effectiveness of the treatment combinations will be determined on one-acre sites. In the unreplicated test, sites ranging in size from 20 to 400 acres will be treated with various combinations of agents (e.g. Cercospora - Sameodes) and application scenarios (e.g. Cercospora applied in different seasons and in multiple applications) that would be difficult or impossible to replicate within the time frame and scope of this LSOMT.

Test Organisms

75. The organisms to be evaluated in this test include:

- a. Cercospora rodmanii - Plant pathogen
- b. Neochetina eichhorniae and N. bruchi - Weevils
- c. Sameodes albiguttalis - Moth
- d. Arzama densa - Moth

Details of the biology and life history of these organisms are presented in Appendix A.

Replicated Tests

76. Statistical design. To determine the effect of climate on efficacy of the various treatments, a complete random-block design will be used. Each treatment will be replicated three times in each of the two blocks (north and south Louisiana), as follows:

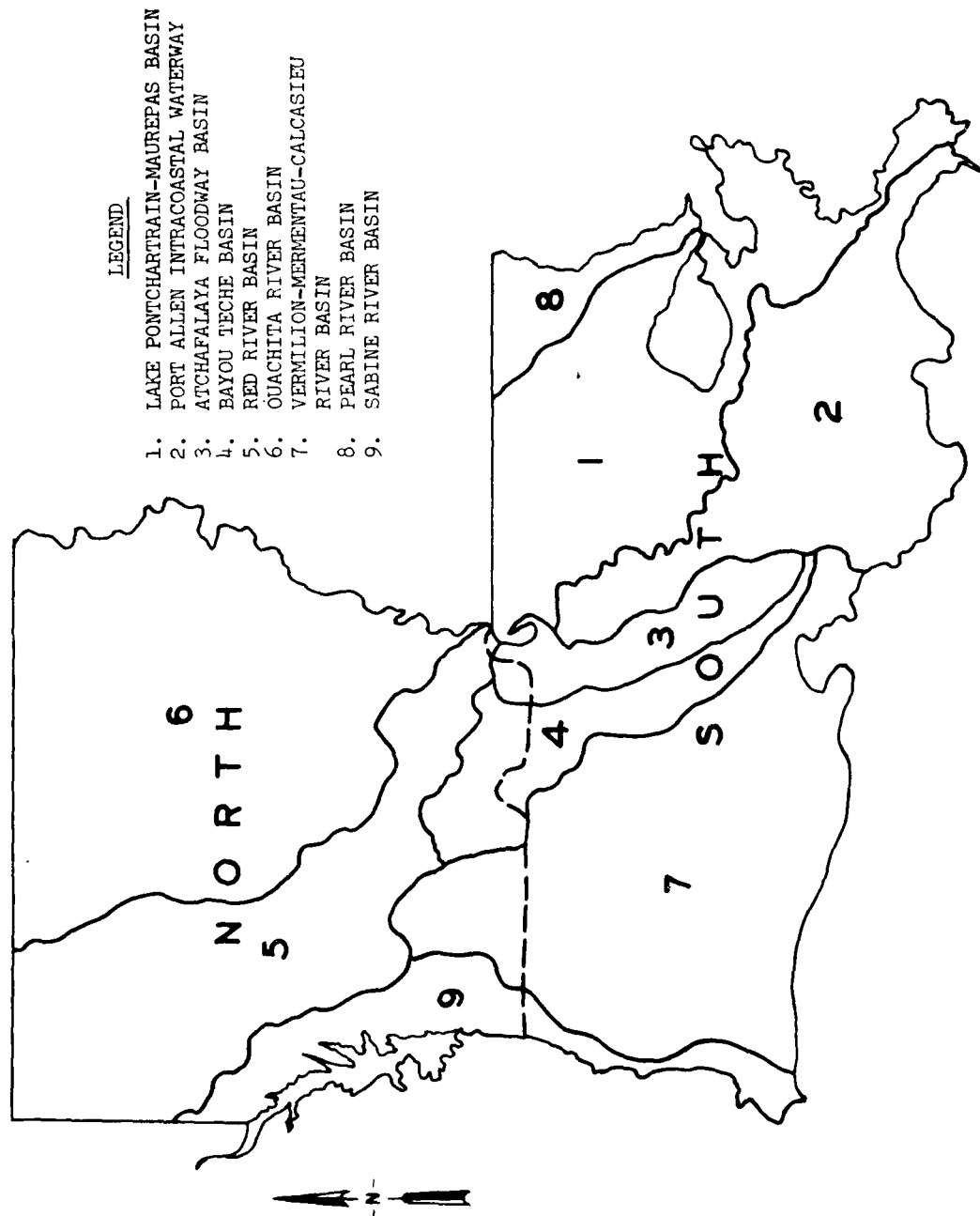


Figure 3. Map of Louisiana showing major river basins and north-south dividing line used in this study

<u>Treatment</u>	<u>North Louisiana</u>	<u>South Louisiana</u>
<u>Cercospora</u> only	3 sites	3 sites
<u>Neochetina</u> only	3 sites	3 sites
<u>Cercospora</u> - <u>Neochetina</u>	3 sites	3 sites
untreated controls	3 sites	3 sites
	<hr/> 12 sites	<hr/> 12 sites

77. Selection of test sites. As stated in paragraph 73, the overall test area consists of the state of Louisiana. For the purposes of this test, the state was divided into north and south regions by using the northern boundaries of the parishes of Beauregard, Allen, Evangeline, St. Landry, and Point Coupee as the dividing line (Figure 3). The resulting line generally divides the state of Louisiana topographically and climatologically. Such a delineation will permit evaluation of the effects of different climatic regimes on the activity of the biocontrol agents. To maximize the potential impact of Cercospora in the overall test area, at least one test site will be utilized in each major river basin. Because a major goal of this study is to determine the epidemiology of Cercospora, not all waterhyacinths in the state will receive direct application of Cercospora during the test period. However, the selection of at least one test site in each major river basin will potentially expose the majority of the waterhyacinth population in Louisiana to the pathogen. With input from New Orleans District and Louisiana Wildlife and Fisheries personnel, whose job it is to control in excess of one million acres of waterhyacinth in Louisiana, sixty potential test sites have been identified (Figures 4 and 5 and Table 3). Numbers and letters for each site in Figures 4 and 5 correspond to the name listed by the same number or letter in Table 3. Each site will be evaluated for possible use in the test and the final selection of test site locations will be made by applying the following test site criteria:

- a. All treatment sites must be of operational interest to NOD.

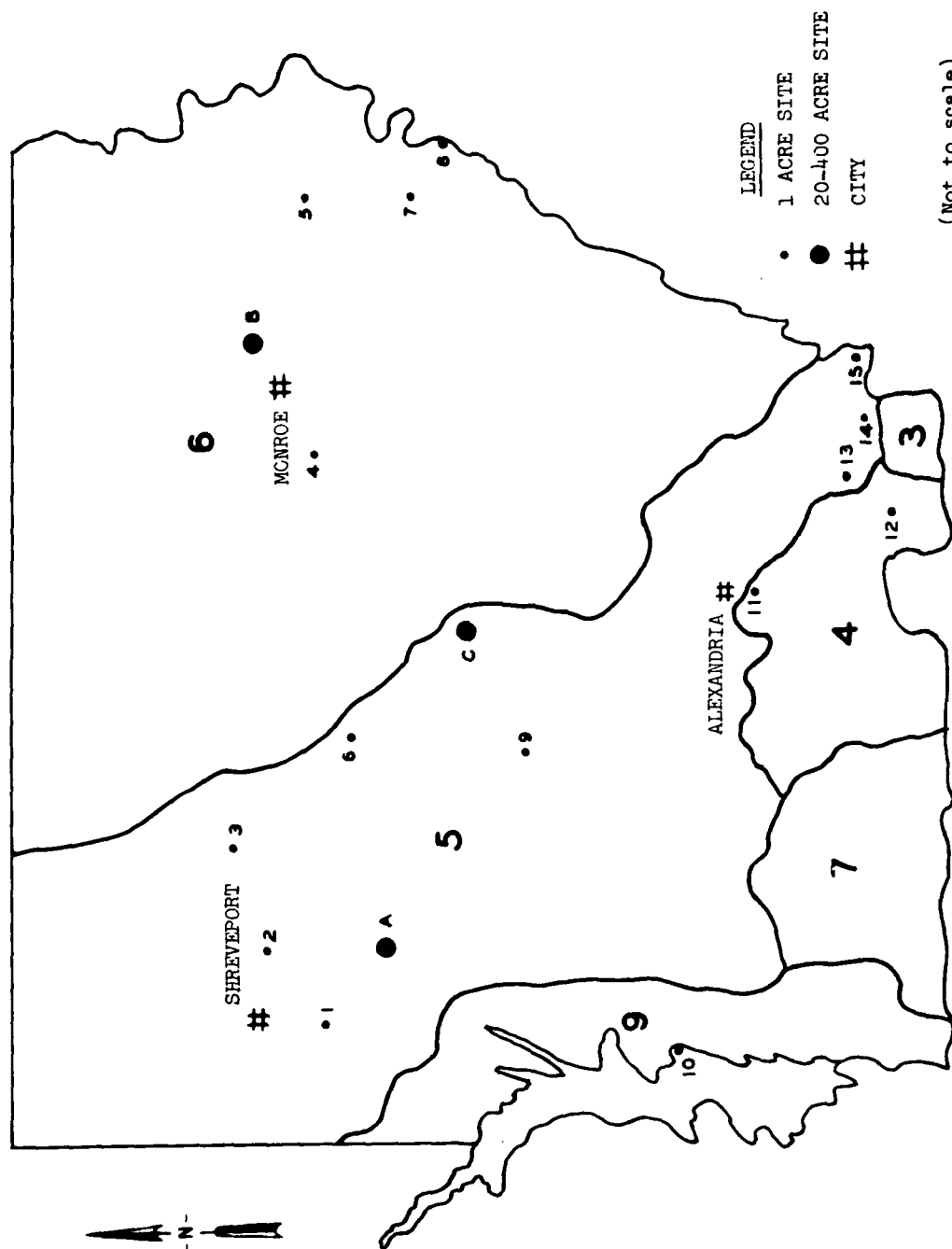


Figure 4. Map of northern Louisiana showing location of potential test sites in relation to major river basins

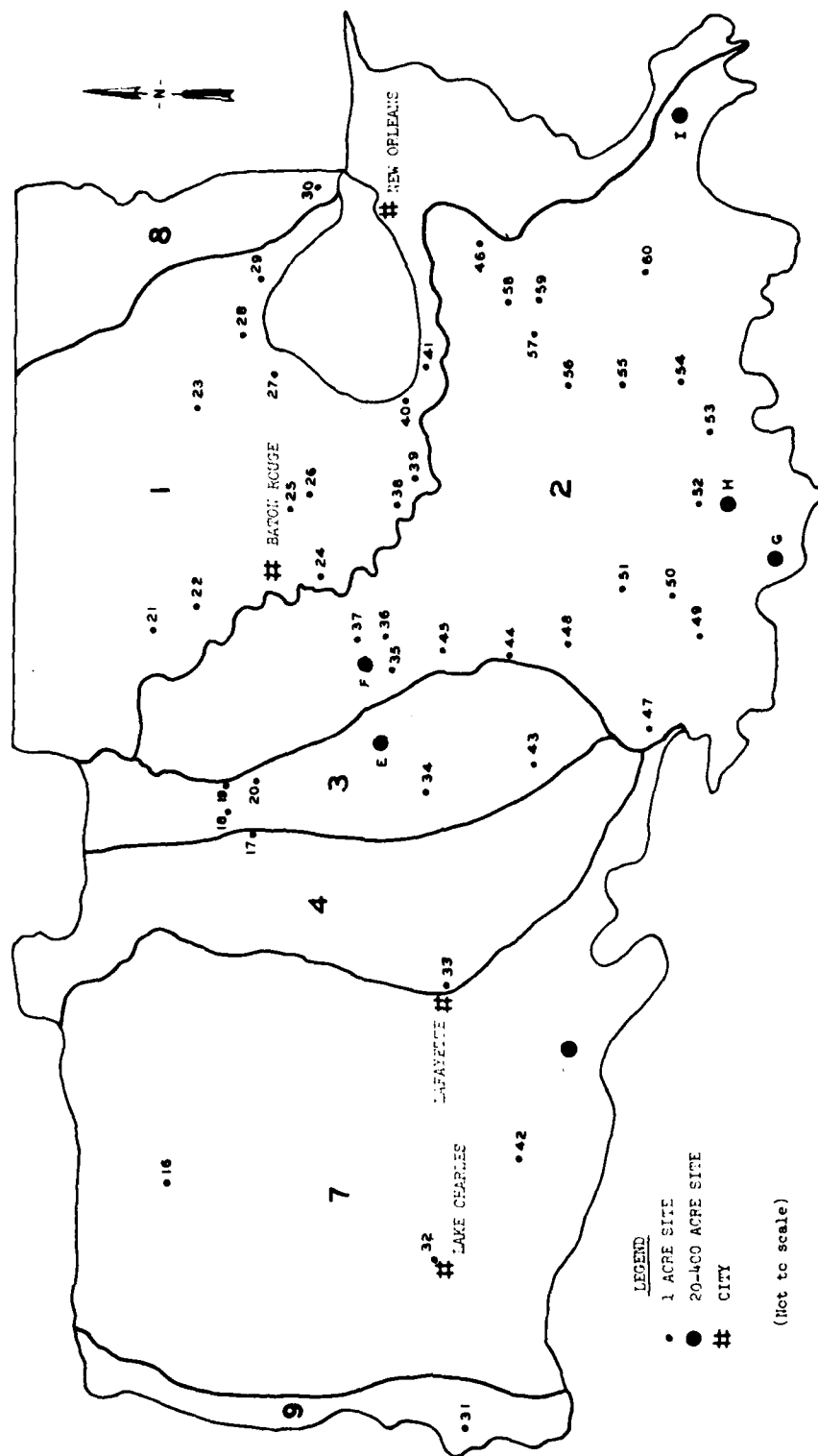


Figure 5. Map of southern Louisiana showing locations of potential test sites in relation to major river basins

Table 3. Potential Sites for
Large-Scale Field Application Test

Replicated Test Sites

<u>Site #</u>	<u>Site Name</u>	<u>Parish</u>
1	Wallace Lake	Desoto
2	Meyers Lake	Bossier
3	Lake Bisteneau	Webster
4	Chinnie Lake	Oudchita
5	Bayou Macon Cutoff	Franklin
6	Saline Lake	Natchitoches
7	Gunby Dam	Tensas
8	Lake St. Joe	Tensas
9	Old River Cutoff	Natchitoches
10	Negreet Creek	Sabine
11	Bayou Roberts	Rapides
12	Bayou near Bunkie	Avoyelles
13	Spring Bayou	Avoyelles
14	Hamburg Loop	Avoyelles
15	Hwy 15 Pits	Concordia
16	Bundicks Lake	Beauregard
17	Two O'clock Bayou	St. Landry
18	Junction Pits	St. Landry
19	Hays Pits	St. Landry
20	Krotz Spring Pits	St. Landry
21	False Bayou	Point Coupee
22	Grand Bay Lake	Point Coupee
23	Hwy I-55 Pits	Tangipahoa
24	Spanish Lake	East Baton Rouge
25	Port Vincent	Livingston
26	French Settlement	Livingston
27	Middle Bayou	Tangipahoa
28	Shell Hole	Tangipahoa
29	Medisonville Swamp	St. Tammany
30	Salt Bayou	St. Tammany
31	Black Bayou	Cameron
32	English Bayou	Calcasieu
33	Lake Martin	St. Martin
34	Dautrive Lake	St. Martin
35	Bayou Sorrel Pits	Iberville
36	404 Canal	Iberville
37	Pats Bay	Iberville
38	Grammercy Canals	Ascension
39	Grammercy Pit	Ascension
40	La Place Pit	St. John the Baptist
41	Bayou La Branch	St. Charles
42	Willow lake	Cameron
43	Charenton	St. Mary
44	North Bell River Pits	Assumption
45	Bayou Pigeon Pits	Assumption
46	Jefferson Parish Canal	Jefferson

Table 3. (concluded)

<u>Site #</u>	<u>Site Name</u>	<u>Parish</u>
47	Berwick Pits	St. Mary
48	Lake Verrett	Assumption
49	Bayou Chene	Terrebonne
50	Hwy 90 Canal	Terrebonne
51	Hwy 398 Canal	Assumption
52	Humphrey Canal	Terrebonne
53	Hwy 315 Canal	Terrebonne
54	Bayou Terrebonne	Terrebonne
55	Raceland Canal	Lafourche
56	Lake Boeuf	Lafourche
57	Bayou Des Allemands	Lafourche
58	Paradise Canal	St. Charles
59	Bayou Gauche	St. Charles
60	Lafitte Canal	Jefferson

Unreplicated Test Sites

A	Smithport Lake	Desoto
B	Black Bayou (Monroe)	Ouachita
C	Iatt Lake	Grant
D	Little Pecan	Cameron
E	Lake Henderson	St. Martin
F	Upper Grand River	Iberville
G	Lost Lake	Terrebonne
H	Lake Theriot Canals	Terrebonne
I	Venice	Plaquemines

- b. All treatment sites must have at least seventy-five percent coverage by waterhyacinths at the time of treatment application.
- c. The population of waterhyacinth must be confined to the test site by either natural or artificial barriers. If necessary, temporary retaining structures (e.g. log booms or fences) will be used to stabilize the waterhyacinth population in a given site that is otherwise acceptable as a test site.
- d. Selected sites must not be sprayed with chemical herbicides or otherwise altered during the course of the study. It will be essential that individuals or agencies responsible for aquatic plant management in the potential test site agree to refrain from spraying in these sites for the duration of the waterhyacinth control study.
- e. Sites must not be subject to extreme water level fluctuation or flushing action due to heavy rains or tidal actions.
- f. Sites must conform to other test requirements specified in paragraphs 76 and 77 with respect to location and size of test plots, and population levels of insect agents currently present on the site.

During the spring and summer of 1979, WES personnel will visit and evaluate all potential test sites using the above criteria. By November 1979, all sites will have been chosen, and treatments will be allocated to sites in the following manner: six sites with established Neochetina populations and six sites with no Neochetina will be selected in each region (north and south). Then, three of the sites in each region with and without Neochetina will be randomly selected for treatment with Cercospora. The resulting treatments will conform to the statistical design identified in paragraph 76.

78. Treatment of sites. Treatments will be effected in the following manner:

- a. Neochetina - As a result of the procedure to be used in allotment of treatments (paragraph 77), it will not be necessary to establish Neochetina populations on the test sites. Since 1974, the Aquatic Plant Control section of the Louisiana Wildlife and Fisheries Commission released in excess of 170,000 Neochetina eichhorniae and Neochetina bruchi in over 100 sites in Louisiana, predominately in the southern region (Figure 1). They report healthy, reproducing populations at the release sites and have determined that the insects are radiating out from the release sites. Therefore, the Neochetina spp. are considered to be established in the test area, and sufficient numbers of sites are available for this test. A preliminary survey of sites known to contain Neochetina will be made to assess the population density of the species and to select sites of approximately equal population densities.
- b. Cercospora - The spore formulation of Cercospora will be applied to the selected test sites as soon as possible in the spring of 1980. The exact time of initiation will depend on the rate of recovery of the waterhyacinth from the previous winter, but it is anticipated that treatment application will commence by 1 April 1980 in the southern region. All 12 sites scheduled to receive Cercospora will be treated within 30 calendar days. Abbott Laboratories has agreed to produce sufficient test quantities of the Cercospora spore formulation at the time requested in this test plan. The application rate of Cercospora to be used in the test will be the most effective rate as established by the efficacy test described in paragraphs 43-56. The formulation will be applied in a uniform manner, thereby exposing all waterhyacinth plants in the

test site. Conventional chemical herbicide mixing tanks and spray equipment will be used for application of the formulation. The optimum nozzle for application of the formulation will have been determined in the Cercospora spore formulation pilot field study. Applications will be made by NOD and/or LWLFC personnel from airboats, johnboats, tank trucks, or helicopter, as dictated by the location and conditions of the test site.

79. Permits and precautions. An experimental use permit will be obtained from EPA prior to the application of Cercospora in these field tests. All permits for application will also be obtained from the state of Louisiana and local governments as necessary. All applications of the spore formulation of Cercospora used in these tests will be treated as herbicide applications and will be done by or under the direct supervision of an EPA-certified applicator. The applicator will be required to conform to the guidelines and standards for application as provided by the EPA and the Occupational Safety and Health Administration.

80. Data collection. In accordance with the rationale and approach set forth in paragraphs 69-71, specific parameters of the following major data categories will be monitored in each test site during the test period:

a. Biological

Waterhyacinth population
Test organisms
Other organisms impacting waterhyacinth

b. Physical

General system qualities
Water quality
Meteorology

The specific parameters to be monitored for each major data collection

category are presented in Table 4. In addition to these data, color and/or color IR photography will be employed to provide a visual record of overall changes in the waterhyacinth population in the test sites during the test period. Photographic stations will be established at each test site and photographs will be taken along defined compass settings on each sampling date. All biological and meteorological data included in Table 4, water depth and temperature, and pH will be collected prior to treatment, at 2, 4, 6, 8, 12, and 16 weeks post-treatment, and then quarterly through June 1982. All other parameters identified in Table 4 will be monitored prior to treatment, and at one and two years post-treatment.

81. Data analysis and portrayal. The procedures for data analysis and portrayal will be the same as were used in the Cercospora spore formulation pilot field study.

82. Schedule and coordination. It is anticipated that the WES will contract the monitoring portion of the LSOMT. The scheduling and coordination of the monitoring phases of the experiment will be arranged and maintained by the WES through contacts and conferences with the contractors. Prior to initiation of the work, each contractor will be required to submit a complete data collection and analysis plan to the WES. Upon receipt of these plans, the WES will meet with the contractors and a final data collection plan will be developed that provides for continuity and compatibility of the resulting data. Each contractor will be asked to analyze their own data, but all data will be submitted to the WES for additional analyses.

83. Reporting. All data and preliminary narrative reports, the first of which will include a map showing sampling locations, will be submitted to the WES data management team upon a mutually agreed time schedule, and on a form or in a format specified by the WES. Each contractor will prepare a final report in accordance with WES requirements for publication of contract reports, and this report will be submitted to the WES in a form suitable for direct publication.

Table 4. Summary of Data Collection Program for
LSOMT - Insects and Pathogens for Control of Waterhyacinth

<u>Major Data Categories</u>	<u>Specific Parameters</u>	<u>Sampling Interval</u>
Target Plant (Waterhya- cinth)	Abundance Biomass Reproduction Flowering Plant Condition Height Root Length # of petioles	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
Test Organisms <u>Neochetina</u> spp.	Population Density Population Conditions # of individuals at each stage (adult, larvae, pupae) Spatial Distribution Impact on Target Plant (feeding scars) (larvae tunnels)	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
<u>Cercospora</u> <u>rodmanii</u>	Infectivity (propagules per leaf) Epidemiology (rate of spread) Pathogenicity (leaf damage index)	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
Other Organisms Impacting Target Arthropods (insects & mites)	Population Density Population Condition # of individuals at each stage (adult, larvae, pupae) Spatial Distribution Impact on Target Plant (feeding scars) (larvae tunnels)	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
Pathogens (viral, bacterial, fungal)	Infectivity (propagules per leaf) Epidemiology (rate of spread) Pathogenicity (leaf damage index)	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter

Table 4 (continued)

Major Data Categories	Specific Parameters	Sampling Interval
General System Qualities	Geographic Location	Initially
	Perimeter Description	Initially
	Water Inflow and Outflow	Initially
	Backshore Land Use	Initially
	Water Depth	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter
Water Quality	Water Temperature	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter
	pH	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter
	Dissolved Oxygen	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter
	Hardness	Annually
	Total Phosphorus	Annually
	Total Organic Nitrogen	Annually
	Nitrate-Nitrite	Annually
	Potassium	Annually
	Ammonia	Annually
	Salinity	Annually
Meteorology	Rainfall	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter
	Air Temperature	Pretreatment, 2,4,6, 8,12,16 weeks post-treatment, then quarterly thereafter

Table 4 (concluded)

Major Data Categories	Specific Parameters	Sampling Interval
Meteorology (con't)	Frost Days	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
	Relative Humidity	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter
	Wind Velocity and Direction	Pretreatment, 2,4,6, 8,12,16 weeks post- treatment, then quarterly thereafter

Unreplicated Tests

84. Purpose. The purpose of these tests is to demonstrate the effectiveness of combinations of biocontrol agents and application scenarios when applied at a scale (20-400 acres) that is not feasible for replication. Such applications will provide not only valuable data for use in development of an operational plan, but also a test of the effectiveness of previously untested agent combinations (e.g. Cercospora - Sameodes) or application scenarios (multiple applications of Cercospora).

85. Objectives. Specific objectives of the unreplicated tests include:

- a. To determine the effectiveness of the spore formulation of Cercospora when applied in summer or fall.
- b. To determine the effectiveness of multiple applications of the spore formulation of Cercospora.
- c. To determine the effectiveness of a Cercospora - Arzama agent combination in controlling waterhyacinth.
- d. To establish Sameodes albiguttalis in Louisiana.
- e. To determine the effectiveness of a Cercospora - Sameodes agent combination in controlling waterhyacinth.

86. Test scenarios. Six separate tests will be conducted during the LSOMT, as follows:

- a. Cercospora applied in summer
- b. Cercospora applied in fall
- c. Multiple application of Cercospora (spring, summer, and fall)
- d. Cercospora - Arzama
- e. Sameodes
- f. Cercospora - Sameodes

All Cercospora applications will be made in the spring, except where otherwise noted. In addition, other organisms, combinations of organisms, or application scenarios may be tested at this scale. Likewise, one or

more of the above tests may be excluded, if circumstances warrant such action. For example, if no naturally occurring Arzama population of sufficient density can be found, the Cercospora - Arzama test will be excluded.

87. Selection of test sites. Potential sites for the unreplicated tests have been identified (Figures 4 and 5). Letters for each site identified in Figures 4 and 5 correspond to the name listed by the same letter in Table 3. Other potential sites may be considered prior to final selection of test sites. Each site will be evaluated for possible use in the test, and the final selection of sites will be made by applying the test site criteria described in paragraph 77. During the spring and summer of 1979, WES personnel will visit and evaluate all potential test sites. By November 1979, all sites will have been chosen and treatments will be allocated to sites for each particular scenario.

88. Treatment of sites. The following procedures will be used to effect treatment of the test sites:

- a. Cercospora - Three of the specified tests involve only the application of the spore formulation of Cercospora. These tests will differ only in the timing and number of applications. In one test Cercospora will be applied initially in the spring, followed by repeated applications in mid-summer and early fall. In separate tests, Cercospora will be applied to one site in mid-summer and to another site in early fall. In each case, the application rate of Cercospora to be used in the test will be the most effective rate as established by the efficacy test described in paragraphs 43-56. Abbott Laboratories will be responsible for supplying the spore formulation at the time and in the amount required for the test. The formulation will be applied in a uniform manner, thereby exposing all waterhyacinth plants in the test site to

the pathogen. The formulation will be applied by helicopter, using appropriate spray equipment.

- b. Cercospora - Arzama - One site that has a high population density of Arzama will be selected to receive a Cercospora treatment. Arzama is a native moth that occurs at large population densities in localized areas in Louisiana (Appendix A). Consequently, sites known to have periodically experienced high population densities of Arzama will be inspected, and one that has abundant Arzama larvae will be treated with the same rate of Cercospora and by the same method as was used in the other unreplicated tests. The site and timing of this application will be selected by WES personnel.
- c. Sameodes - Sameodes albiguttalis is an exotic moth which has recently been released from quarantine by USDA and is not established anywhere in the test area (Appendix A). Following the selection of two suitable sites using the previously described procedures, populations of Sameodes will be established beginning in June 1979. The brood stock will be furnished from the populations currently being maintained at WES and USDA in Ft. Lauderdale, Florida. Procedures to be used for establishing the field populations will be based on recommendations from USDA entomologists at the Aquatic Plant Management Laboratory, Ft. Lauderdale, Florida. Based on preliminary results of the studies currently underway by USDA, it is anticipated that at least one year will be required to establish Sameodes in Louisiana.
- d. Cercospora - Sameodes - When it has been determined that Sameodes has become established in the test sites, Cercospora will be applied to one of the sites using the same procedures as for the other unreplicated tests. The determination of Sameodes establishment and the proper timing for application of Cercospora will be made by WES personnel.

89. Data collection, analysis, and portrayal. The basic parameters, procedures, and timing for data collection, analysis, and portrayal will be the same as were used in the replicated test (paragraphs 80 and 81). Major data categories and frequency of sampling will be the same for each test. On-site monitoring will be accomplished by WES personnel. In addition to the previously described procedures, semi-annual remote-sensing missions will be used to monitor changes in the waterhyacinth population. Photomissions will be flown in April and November of each year for each test site. Requirements for the design of the remote-sensing missions are described by Long (12).

90. Reports. Annual progress reports will be prepared and submitted for review. A final report will be prepared by 31 March 1983 (Table 5).

Supportive Studies

91. During the course of the preliminary and exploratory studies leading to the formulation of this LSOMT, several problems were revealed relating to aspects of the biology of the organisms which directly affect their value as biocontrol agents for waterhyacinth or on eventual management of them for that purpose. While these problems are not essential to a successful conclusion of the LSOMT, they are nonetheless relevant to its purpose, and are within its defined scope. Some of the most important of these problems are listed here as supportive studies, with the intention that they will be addressed under the scope of this LSOMT.

Covered Plot Studies of Plant-Insect-Pathogen Interactions

92. Objective. The objective of this study is to elucidate the relative contribution of the various organisms and combinations of organisms to the decline of the waterhyacinth. The study will be conducted in an environment that will prevent the uncontrolled spread of the test agents among the test plots.

Table 5. Schedule of Tasks and Events Included in the LSOMT

	FY 79	FY 80	FY 81	FY 82	FY 83
	F M A M J J A S	O N D J F M A M J J A S	O N D J F M A M J J A S	O N D J F M A M J J A S	O N D J F M
TASKS					
I. BACKGROUND STUDIES					
Lake Concordia Final Report (WES) ^a		X			
II. PILOT FIELD STUDIES (WES)					
A. Monitoring of Sites					
B. Interim Report (initiation to completion)					
C. Final Report (initiation to completion)					
III. CERCOSPORA FORMULATION TEST					
A. Laboratory Efficacy Test (WES)					
1. Establishment of Test					
2. Monitoring Period					
3. Final Report (initiation to completion)					
B. Small-Scale Field Application (WES)					
1. Establishment of Test Plots					
2. Monitoring Period					
3. Final Report (initiation to completion)					
IV. LARGE-SCALE FIELD APPLICATION TEST (WES, NOD ^b , LWLFC ^c)					
A. Identification of Potential Test Sites					
B. Inspection of Potential Test Sites (WES)					
C. Selection of Test Site (WES)					
D. Request for Proposal for Monitoring of Sites (WES) (initiation to completion)					
E. Selection of Contractors (WES)					
F. Establishment of <u>Sameodes</u> (WES, USDA)					
G. Application of <u>Cercospora</u> (WES, LWLFC, NOD)					
H. Monitoring of Sites (Contract)					
I. Contractor Final Reports to WES					
J. Final Report (initiation to completion)					
V. SUPPORTIVE STUDIES (WES, Contract)					
VI. SUMMARY REPORT (WES)					

^aWES - Waterways Experiment Station

^bNOD - New Orleans District

^cLWLFC - Louisiana Wildlife and Fisheries Commission

93. Relevance. The study bears directly on the problem of defining efficacious application strategies, with respect to determining circumstances in which applications of an organism might effect a more satisfactory rate or degree of control on a waterhyacinth population.

94. Status. There has been little previous work directly responsive to this need (13), and that work was not definitive for the organisms now thought to be most promising for biocontrol of waterhyacinth.

95. Proposed action. Pilot studies will be initiated in-house in 1979. Self-contained tanks, covered separately with screen, cloth, or plastic to prevent cross-contamination, will be used as the test plots for this experiment. Although there are potentially very many combinations of organisms that may be included in this experiment, the initial test organisms will be Cercospora, Neochetina, and Sameodes. Also, it is understood that ambient environment (temperature, water quality, etc.) may affect the responses of the organisms in the proposed relations. However, the pilot studies will be conducted in one environment that is sufficient for the growth of waterhyacinth. Results of this in-house pilot study will be evaluated, and a determination will be made as to whether further tests are needed. The results and recommendations will be presented in a report. If further tests seem justified, the needs will be outlined in that report. Subsequently, an appropriate test plan or Request for Proposal (RFP) will be submitted for continuation of the studies.

Epidemiology and Etiology of Cercospora Disease

96. Objective. To elucidate the mode of action of Cercospora including the dispersal mechanisms, its means of entry into the hyacinth plant, and its anatomical and physiological interactions with the host plant, with special reference to its disease-producing function.

97. Relevance. Understanding the mode of dissemination and infection of the fungus has immediate and direct relevance to optimum management of the organism, both with respect to application techniques and deployment strategies. In addition, understanding the mode of entry and other aspects of its symbiosis with the host will contribute to understanding the role of this organism in a mixed-agent biocontrol system, and thereby provide a rational basis for the design of such systems.

98. Status. Work on this problem has been initiated by the University of Florida (UF) under contract to the APCRP for studies of pathogens on aquatic plants. Through this work, some aspects of the problem have been addressed, but the contract will expire in September 1979, and many important questions on the epidemiology and etiology of the disease could remain unanswered.

99. Proposed action. Action on this study will be postponed until results of the work by the UF are available for evaluation. Depending upon those results, one or more RFP will be prepared to address additional aspects of the problem.

Requirements for Artificial Rearing of Arzama

100. Objective. The objective is to develop methods for culturing Arzama densa in an artificial environment for timed mass releases in the field.

101. Relevance. In view of the observed destructiveness of the individual Arzama larvae on waterhyacinth, it would be advantageous to release this insect en masse on sites where the waterhyacinth is insufficiently impacted by naturally regenerating populations of other insects or pathogens, or in season unfavorable for the natural regeneration of this or other insects in the field. Further, early season Arzama releases before the waterhyacinth has attained significant biomass may inflict proportionally greater damage to the waterhyacinth. This could result in greater advantage to Neochetina and Cercospora.

102. Status. This work is currently under way through a funding agreement between the WES and the USDA Southern Weed Science Laboratory, Stoneville, Mississippi.

103. Proposed action. The work at Stoneville will be continued through FY 79. At that time, the results of the work will be reviewed and a decision will be made on the need for further pursuit of this problem.

PART IV: MANAGEMENT IMPLICATIONS OF TEST RESULTS

104. The objective of the LSOMT is to determine if any of the organisms being evaluated in this test, either alone or in combination, are operationally feasible as a biological control for waterhyacinth. Feasibility in this context is meant to imply that the use of these organisms is effective, practical, economically acceptable, and environmentally compatible. From an operational standpoint, there eventually must be a management program for continual, operational maintenance to ensure continued control wherever waterhyacinth is established. This research is intended to provide operations management with the information necessary to determine:

- a. Situations amenable to waterhyacinth control using these organisms.
- b. Degree and type of application necessary to maintain the waterhyacinth population at a desired level in any particular system.
- c. The type and number of facilities required to maintain a sufficient supply of organisms to support a District-wide waterhyacinth control program.
- d. The manpower, equipment requirements, and logistic problems of sustaining such a control program.
- e. Permit regulation requirements for proper compliance with governing agencies.
- f. Requirements for periodic monitoring of established systems.

Data addressing these and other requirements will be used in the development of an engineering manual for the operational use of the test organisms in the biological control of waterhyacinth in the NOD.

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APPENDIX A: TEST ORGANISMS

Introduction

1. The purpose of this appendix is to provide background information on the organisms mentioned in the LSOMT test plan. For each species, it includes a brief discussion of:

- a. Taxonomic status
- b. Life cycle
- c. Feeding behavior and host specificity
- d. Preliminary research of biocontrol potential

The organisms are presented in the following order: Cercospora, Acremonium, Neochetina eichhorniae, Neochetina bruchi, Sameodes, Arzama, and Orthogalumna.

Cercospora rodmanii Conway

Taxonomy

2. A pathogen that produced a leaf spot disease was isolated from declining waterhyacinth in Rodman Reservoir, Florida, in 1973 (6). The isolate was originally thought to be Cercospora piaropi Tharp (14), a widespread, well-established species that is slightly pathogenic on waterhyacinth. However, a thorough review of discrepancies in symptomology and conidial morphology resulted in the isolate being described as Cercospora rodmanii Conway (Fungi Imperfecti) (6).

Life Cycle

3. Being a member of the form class Deuteromycetes (Fungi Imperfecti), the sexual stage of the species is unknown. Conidia, which are asexual spores, are produced in lesions of waterhyacinth leaves and petioles, and are normally disseminated by wind. Conidia that land on waterhyacinth leaves germinate, enter the leaf through stomates or other openings (e.g. wounds), and spread by mycelial growth into intercellular spaces. Mycelium growth will continue, eventually infecting the entire leaf and petiole.

Symptoms

4. The symptoms of the leaf spot disease caused by C. rodmanii include small, punctate leaf spots, chlorosis of the leaf and petiole, general coalescence of the leaf spots, and rapid necrosis of the leaf blade (15). The disease spreads rapidly in suitable environments (16).

Host Specificity

5. C. rodmanii has been shown to be specifically pathogenic to waterhyacinth. In a test to determine the host range of the species, 85 economically or ecologically important taxa (58 species representing 22 families) were exposed to C. rodmanii in both greenhouse and field tests. C. rodmanii showed no pathogenic tendencies toward any species except waterhyacinth. It was concluded that C. rodmanii would not pose a threat to other vegetation when applied as a control for waterhyacinth (17).

6. The potential toxicity or pathogenicity of C. rodmanii to animals has been or is currently being investigated. Studies in which mosquito fish (Gambusia affinis) were exposed to C. rodmanii led to the conclusion that the species is non-toxic and nonpathogenic to fish (18). Abbott Laboratories is currently conducting similar tests using laboratory animals to determine toxicity or pathogenicity of C. rodmanii to animals. When the tests have been completed, the results will be included as part of a petition to the Environmental Protection Agency for an Experimental Use Permit (EUP). As expected, preliminary data indicate that C. rodmanii has no adverse effect on mammals. Based on these studies, C. rodmanii is expected to have no direct effect on the nature flora and fauna of the state of Louisiana.

Acremonium zonatum (Sawada) Gams

Taxonomy

7. In August of 1971 Rintz found a zonal leaf spot occurring on waterhyacinth in central Louisiana and shortly thereafter in north-central

Florida (19). Isolates from both areas produced identical fungi, which was originally identified as Cephalosporium zonatum. Subsequent studies by Gams (20) resulted in the reassignment of the organism to the genus Acremonium, to be designated A. zonatum (Sawada) Gams.

Life Cycle

8. A. zonatum is a member of the form class Deuteromycetes (Fungi Imperfecti). Oval, uni-cellular conidia occur singly from the apex of phialides and collect in mucilaginous heads. The conidia are disseminated by direct contact of plant tissues or passively transported to new plant tissues by bird and insects. The conidia germinate and enter leaf tissues through stomates or other openings (e.g. wounds) and spread by mycelial growth into intercellular spaces (19).

Symptoms

9. The disease is first evident as small sunken lesions on the leaf surface and petioles. The infected areas gradually enlarge and coalesce, becoming distinctly zonate with light brown bands alternating with narrower dark brown bands. Lesions are oval to irregular in shape with the surface often covered with a waft of cottony mycelium. The lesions continue to enlarge until the leaf dies or conditions become unfavorable for disease development (21).

Host Specificity

10. Plants representing 12 families were tested against A. zonatum to determine its host specificity. The results of these tests indicated that A. zonatum can infect a wide range of host plants. Yet, despite this wide host range, it apparently attacks only the fig (Ficus sp.) in North America and it is not seriously pathogenic on that species (21). It was therefore considered a safe candidate for biocontrol of waterhyacinth.

Neochetina eichhorniae Warner

Taxonomy and Description

11. Neochetina eichhorniae Warner (Coleoptera: Curculionidae, Bagoini) has been introduced into the U. S. as a potential agent for control of waterhyacinth (22,23). It is commonly known as the mottled waterhyacinth weevil. The genus Neochetina is comprised of four closely related species, N. eichhorniae, N. bruchi Hustache (see paragraphs (16-19), N. affinis Hustache, and an un-named species. The species are distinguished by size, rostrum features, and genitalia (23).

Life Cycle

12. Adults of N. eichhorniae feed on the leaves (pseudolamina) of waterhyacinth, and deposit eggs in feeding scars or other wounds. After 7-10 days, larvae emerge and tunnel downward in the petiole toward the crown of the plant. After three instars (approximately 69 days), the larvae enter the root system, form a cocoon from secondary roots of the waterhyacinth plant, and pupate. After 14 days, the new adults emerge and begin to feed almost immediately. The adult lifespan averages about 60 days (24). The total generation time is 120 days (23).

Host Specificity

13. N. eichhorniae is host specific to waterhyacinth, being unable to complete its life cycle on other plants (25). This is probably due primarily to the interrelationship between N. eichhorniae and waterhyacinth during the pupal stage (23).

Preliminary Studies

14. The history of discovery, introduction, and quarantine tests of N. eichhorniae is reviewed by Spencer et al. (25) and Perkins (26). N. eichhorniae has been introduced to the U. S. under quarantine by the USDA Biological Research Laboratories, has undergone the required specificity tests, and with permission from the USDA-USDI Weed Committee Working Group on Biological Control of Weeds, has been released in selected areas in Louisiana and Florida.

15. Its effectiveness as a control agent for waterhyacinth in the U. S. derives from three factors (26):

- a. larval feeding and tunneling;
- b. adult feeding; and
- c. bacterial and fungal decay associated with the feeding wounds.

It was thought that these effects should be enhanced with the higher population densities that were expected to occur in the U. S. in the absence of parasites and competitors that tend to suppress populations in their native Argentina, and the effect of bacterial and fungal decay was expected to be enhanced in proportion to the excessive feeding.

Neochetina bruchi Hustache

Taxonomy and Description

16. The taxonomic relationship of N. bruchi and related species is presented in paragraph 11. It is commonly called the chevroned waterhyacinth weevil, due to the presence of a conspicuous light area on the elytra. It is slightly larger than N. eichhorniae (22,23).

Life Cycle

17. The life cycle of N. bruchi is very similar to that of N. eichhorniae (see paragraph 12). However, the generation time for N. bruchi is considerably reduced (96 days) than for N. eichhorniae (120 days) (23,27,28).

Host Specificity

18. Although N. bruchi is not as host specific as N. eichhorniae, data presented by Perkins and Maddox (29) clearly indicate that N. bruchi is safe for release on waterhyacinth. In controlled tests, the adult insect fed slightly on Pontederia cordata and Eichhorniae azurea (Pontederiaciae) and Commelina virginica and Tradescantia elongata (Commelinaceae). However, the insect did not complete its life cycle on

these plants. The Working Group on Biological Control of Weeds has approved the release of N. bruchi in the United States.

Preliminary Studies

19. N. bruchi has been released in Louisiana and Florida. However, the exact effect of this species has been impossible to determine because it has intermingled with N. eichhorniae to form a mixed population of Neochetina.

Sameodes albiguttalis (Warren)

Taxonomy

20. Sameodes albiguttalis (Pyralidae), commonly called the Argentine waterhyacinth moth, is a member of large family of small, undistinguished moths (30). The larvae of most pyralid moths are foliage feeders or stem or root borers, one of the more important species being the European Corn Borer. S. albiguttalis is known only from Trinidad and South America and is recorded only from Eichhornia crassipes (30). The adults can be distinguished by color, with the male exhibiting a brownish color and the female a lighter, yellowish color.

Life Cycle

21. S. albiguttalis is multivoltine with the mean oviposition per female about 200. The life cycle is a complete metamorphosis with the duration of each stage as follows: egg - 6 days; larvae (6 instars) - 20 days; and pupa - 8 days. The total generation time averages 35 days and the adults die shortly after mating and oviposition.

Host Specificity

22. S. albiguttalis was tested against 34 species in Argentina and under quarantine in the United States for host specificity and was found to only reproduce on waterhyacinth. In starvation tests, S. albiguttalis fed slightly on Pontederia cordata, but did not reproduce on this species (31).

It has also been tested against 50 species in Australia, and was found to reproduce only on waterhyacinth (31). These data were used to obtain clearance for its release in the United States in Sep 1977.

Preliminary Studies

23. The initial release of S. albiguttalis was made in southern Florida as part of a research project conducted by Dr. Ted Center, USDA Aquatic Plant Management Laboratory, Ft. Lauderdale, Florida. S. albiguttalis has been successfully established in four areas (32). They are presently studying these populations dynamics and rate of dispersal. Until these tests and others described in this document are concluded, Sameodes will not be available for general operational use.

Arzama densa Walker

Taxonomy

24. (Lepidoptera: Noctuidae (Amphiprynae)). The moth family Noctuidae is a complex group comprising the army-worms, cutworms, and their allies. The taxonomy of the Arzama and related genera was reviewed critically by Center (33), who concluded that "these species [of a complex] probably represent one genus, and the proper name of A. densa Walker is Bellura densa (Walker)." However, because of the widespread current use of the former name and the absence of a definitive study in the literature, he preferred to use the name A. densa Walker. That usage has been continued in the waterhyacinth biocontrol research program.

Life Cycle

25. The adult A. densa lays up to 225 eggs in masses on the upper surface of the waterhyacinth leaves. The eggs are usually in masses of 20-35, and are covered by a yellowish or tan secretion that may include body hairs. After about 15 days, the larvae emerge and begin mining the leaves and petioles. By the time the larvae is in the sixth instar stage, it has reached the crown. The seventh instar larva extensively

damages the crown and rhizome of the waterhyacinth plant. The entire larval period lasts about 160 days. Pupation occurs in the petiole and requires about 18 days. Because of the long larval period, there are only two generations per year (33, 34).

Host Specificity

26. The larvae of Arzama are root and stem-borers of various aquatic plants, and A. densa, as recognized here, is specific to the family Pontederiaceae (33). The species was apparently present in the U. S. before waterhyacinth was extensively naturalized in the country, and is presumed to be native. It feeds extensively on pickerelweed (Pontederia cordata L.), a native aquatic plant closely related to waterhyacinth, and this was presumably its principal host in the U. S. prior to introduction of the waterhyacinth.

Preliminary Work

27. Arzama densa occurs naturally and abundantly in Louisiana (35), and in Florida (36). Its life cycle and its potential effectiveness as a control agent for waterhyacinth were studied by Vogel and Oliver (34, 35), who concluded that it would have significant potential for this purpose if biotic factors (insects and diseases) that reduce its field populations could be reduced so that populations of greater than normal density could develop. They suggested that natural field populations might be supplemented with laboratory-reared larvae if a satisfactory method for rearing could be devised. Methods for rearing and transplanting Arzama densa were tried by Center (33), but that work met with indifferent success. The techniques that were used in that work were inefficient in terms of numbers of insects successfully transplanted in relation to the numbers of eggs started and the required effort. Nonetheless, successful transplants were made for experimental purposes, and a relatively large number of insects were reared and apparently successfully transplanted to the Lake Concordia experiment plots described in the text of this test plan (1,2,3). The moth may have already been present in that area, however, and part of the apparent

dispersal of the insect on those plots may have been from the native population. Following the work by Center (33), and in view of the results on Lake Concordia, work on artificial rearing of this insect for mass release was temporarily suspended. However, the sporadic distribution of the insect, both in space and time, raised questions concerning the behavior as a function of the biochemical relations between it and its host plants. Studies relating to this aspect of the insects' behavior are in progress, as mentioned in Part VIII of this test plan.

Orthogalumna terebrantis (Wallwork)

Taxonomy

28. Orthogalumna terebrantis (Wallwork) (Acari: Galumnidae, Cryptostigmata), commonly called the waterhyacinth mite, is an arachnid and not an insect like the other arthropods previously mentioned (37). It is native to the western Hemisphere and was first described from Uruguay (38).

Life Cycle

29. The eggs are slightly yellowish and require 7-8 days to mature. The small whitish larvae feed in the areas between vascular bundles and produce feeding galleries. The larval development requires 15 days for completion. Adults emerging from the galleries are dark brown to black and are approximately 0.5 mm in length. The female lays eggs in the arenchyma tissue of the leaf, usually one per puncture hole. There are three generations of mites per year (39).

Host Specificity

30. O. terebrantis is highly specific to waterhyacinth. It feeds significantly only on waterhyacinth and eggs were laid only in waterhyacinth in a study by Cordo and Deloach (40). However, waterhyacinth mites have been known to overwinter on waterlettuce (Pistia stratiotes) (39).

Preliminary Studies

31. The waterhyacinth mite has been found in Louisiana (41), and was probably introduced when waterhyacinth was introduced nearly 100 years ago. Life cycle and host specificity studies have been conducted (39,40) and the interactive effect between O. terebrantis and N. eichhorniae on waterhyacinth was studied by Delfosse (42). He found a substantial reduction in the average size and density of the waterhyacinth plants infested by both species. He also found that the waterhyacinth mite was not antagonistic toward the weevil.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Sanders, Dana R

Test plan for the large-scale operations management test of insects and pathogens for control of waterhyacinth in Louisiana / by Dana R. Sanders ... [et al.]. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1979.

iii, 61, 10 p. : ill. ; 27 cm. (Instruction report - U. S. Army Engineer Waterways Experiment Station ; A-79-1)

Prepared for U. S. Army Engineer District, New Orleans, New Orleans, La., and Office, Chief of Engineers, U. S. Army, Washington, D. C.

References: p. 58-61.

1. Aquatic plant control. 2. Insects. 3. Management methods. 4. Pathogens. 5. Water hyacinths. I. United States. Army. Corps of Engineers. II. United States. Army. Corps of Engineers. New Orleans District. III. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Instruction report ; A-79-1.

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